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(57) Abstract

Antide is the decapeptide, N-Ac-D-S-Nal,D-pClPhe, D-3-Pal, Ser,NicLys, D-NicLys, Leu, Ilys, Pro, D-Ala,NH_2 which is an antagonist of luteinizing hormone releasing hormone (LHRH). This decapeptide, like others of the present invention, has high antiovulatory activity (AOA) and releases negligible histamine. Antide is scheduled for scale-up, safety testing and evaluation in the experimental primate and in clinical medicine. Numerous other peptides having structures related to Antide were prepared and tested. These peptides had variations primarily in positions 5, 6, 7 and 8. Of these, N-Ac-D-2-Nal, D-pClPhe,D-3-Pal,Ser,PicLys,*cis*-DPzACAla,Leu,ILys,Pro,D-Ala-NH_2 was one of the most potent.

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EFFECTIVE ANTAGONISTS OF THE LUTEINIZING HORMONE RELEASING HORMONE WHICH RELEASE NEGLIBLE HISTAMINE

This is a continuation-in-part of U.S. Patent Application Number 088,431 filed August 24, 1987 which is incorporated by reference herein.

Research related to the development of this invention was supported in part by the Contraceptive Branch of the National Institutes of Child Health and Human Development, contract no. NOI HD-6-2938 and to the Robert A. Welch Foundation.

The present invention involves the design, synthesis and use of synthetic analogs of the luteinizing hormone releasing hormone (LHRH). An important achievement involved synthesis of analogs which functioned as antagonists of LHRH, were adequately potent to inhibit ovulation and allowed the release of only negligible amounts of histamine. Since there was no way of reliably forecasting the structure of an antagonist having high potency and very low histamine release, it was necessary to explore diverse approaches to discover a combination of structural features which would yield an antagonist of

LHRH having high potency for ovulation inhibition and very low activity for histamine release.

Various peptides such as substance P, vasoactive intestinal peptide, gastrin, somatostatin, as well as others, are well known to cause the release of histamine from mast cells. These cells are in many tissues, such as skin, lung and mesentery, gingiva, etc. Most cells have granules containing histamine and other mediators of inflammation which can be released by peptides to cause capillary dilation and increased vascular permeability. When it was noted that an antagonist of LHRH, for example [Ac-D-2-Nal^1,D-4-F-Phe^2,D-Trp^3,D-Arg^6]-LHRH, caused edema of the face and extremities when it was administered to rats, it appeared likely that such antagonists, if administered to human subjects as a contraceptive agent, would cause serious edema of the face and elsewhere in the human body. Such side effects would likely prevent the administration of such antagonists to human subjects.

The histamine-containing leukocyte is a basophile which can also release histamine when stimulated by many of the same peptides mentioned above. Basophiles differ biochemically from mast cells and such differences may allow for both predictable and unpredictable histamine release in response to antagonists of LHRH. An antagonist of LHRH, to be used clinically to prevent ovulation, should not significantly release amounts of histamine from either mast cells or basophiles.

The discovery of the side effects such as the edematogenic and anaphylactoid actions of LHRH antagonists made desireable the discovery of new LHRH antagonists which prevented ovulation but did not release significant histamine. These undesireable side effects have been observed in rats, and it is likely that the Food and Drug

Administration would not allow the testing of such antagonists in human subjects.

Karten <u>et</u> <u>al</u>. (4), have reviewed available knowledge on the structural characteristics for potent histamine release by antagonists of LHRH. Some of the most important findings are as follows. A most potent LHRH antagonist in triggering histamine release <u>in vitro</u> involved a combination of strongly basic D-amino acid side chains (Arg or Lys) at position 6 and in close proximity to Arg^8, and a cluster of hydrophobic aromatic amino acids at the N-terminus. Thus, there is no specific amino acid of the ten amino acids which is solely responsible for histamine release. On the contrary, structural features ranging from the N-terminus (the amino acids in the first few positions, 1-4, etc.), and basic amino acids toward the C-terminus (positions 6 and 8) somehow participate in histamine release. Even D-Ala in position 10 has some influence on histamine release, the rationale for which is unclear. By themselves, two basic side chains in close proximity, as in positions 6 and 8, are insufficient alone to impart high release of histamine. The cluster of hydrophobic amino acids at the N-terminus is insufficient alone for high histamine releasing activity. Even a hexapeptide fragment has revealed moderate histamine releasing potency. There seems to be no correlation between antiovulatory potency and histamine release of these antagonists, <u>in</u> <u>vitro</u>.

In perspective, much of the entire chain of such decapeptide antagonists may have influence on histamine release. The same perspective appears to be true, but to different degrees, for high antiovulatory activity. These LHRH antagonists are usually decapeptides which indicates that there are ten variables to adjust for a desired anti-ovulatory activity and ten variables to adjust for

eliminating histamine releasing activity. There are even further variations for each of these twenty variables, the number of possible peptides to design, synthesize and assay becoming incalculable. Presumably, some of the ten variables may be independent for anti-ovulatory activity and histamine releasing activity while some variables may overlap for these two biological activities. This situation poses extraordinary difficulties to solve before an antagonist of high potency for anti-ovulation and very low potency for histamine release could be produced.

Diverse structural changes and combinations of the ten amino acids followed by assays of both anti-ovulation and histamine release activities should be performed in the hope that a potent antagonist essentially free of side effects would be discovered. The synthesis of new amino acids to introduce into the decapeptide chains should also be explored since the commonly available amino acids might not suffice.

In the antagonists prepared according to the present invention, arginine and its derivatives were not utilized. Lysine was converted into derivatives with acyl groups or with alkyl groups on the E-amino group. The amino acid ornithine was acylated or alkylated on the d-amino group. Both the L- and D- forms of lysine and the L-form of ornithine were used in synthesizing these acyl and alkyl derivatives. Structurally related intermediates were also synthesized. All together, many new peptides were synthesized by the basic and minimal concepts of ten variables for anti-ovulation activity and ten variables for histamine release, which may be independent or partially overlapping. On such a basis, the number of such peptides that can be designed becomes overwhelming, and every reasonable priority must be considered to reduce

the number of peptides to be synthesized in the hope that a discovery will be realized.

Certain peptides were synthesized, tested and found to demonstrate advantageous peptides. Among these desireable peptides were the following two.

[N-Ac-D-2-Nal^{1},D-pClPhe^{2},D-3-Pal^{3},NicLys^{5},D-NicLys^{6},ILys^{8},D-Ala^{10}]-LHRH was effective to prevent ovulation and released remarkably little histamine.

[N-Ac-D-2-Nal^{1},D-pClPhe^{2},D-3-Pal^{3},PicLys^{5},D-PicLys^{6},ILys^{8},D-Ala^{10}]-LHRH was twice as effective as the above peptide, and released no more histamine than do "super agonists" of LHRH, which are presently being marketed by several pharmaceutical companies.

These two new peptides, and yet additional related peptides described herein provide acceptable balances of high anti-ovulatory activity and low histamine release for full potential clinical utility.

The present invention involves the preparation and use of decapeptides having antiovulatory activity and with minimal histamine-releasing effects. These decapeptides includes those comprising:

Ser^{4}, PicLys^{5} and D-PicLys^{6};

N-Ac-D-2-Nal^{1}, D-pClPhe^{2}, Ser^{4}, D-PicLys^{5} and Pro^{9};

N-Ac-D-2-Nal^{1}, D-pClPhe^{2}, D-3-Pal^{3}, Ser^{4}, D-PicLys^{6}, Pro^{9} and D-Ala^{10};

N-Ac-D-2-Nal^{1}, D-pClPhe^{2}, D-3-Pal^{3}, Ser^{4}, NicLys^{5}, Pro^{9} and D-Ala^{10};

N-Ac-D-2-Nal^{1}, D-pClPhe^{2}, D-3-Pal^{3}, Ser^{4}, Leu^{7}, Pro^{9} and D-Ala^{10};

N-Ac-D-2-Nal^{1}, D-pClPhe^{2}, D-3-Pal^{3}, Ser^{4}, Leu^{7}, Pro^{9} and D-Ser^{10};

D-pClPhe^{2}, Pro^{9} and D-Ala^{10};

D-pClPhe^{2}, Pro^{9} and Ser^{10};

N-Ac-D-2-Nal^{1}, D-pClPhe^{2}, D-3-Pal^{3}, NicLys^{5}, D-NicLys^{6}, ILys^{8} and D-Ala^{10};

N-Ac-D-2-Nal^{1}, D-pClPhe^{2}, D-3-Pal^{3}, NicLys^{5}, D-NicLys^{6}, ILys^{8} and D-Ala^{10};

N-Ac-D-2-Nal^{1}, D-pClPhe^{2}, D-3-Pal^{3}, PicLys^{5}, D-PicLys^{6}, ILys^{8} and D-Ala^{10};

N-Ac-D-2-Nal^{1}, D-pClPhe^{2}, D-3-Pal^{3}, NicLys^{5}, D-NicLys^{6}, IOrn^{8} and D-Ala^{10};

N-Ac-D-2-Nal^{1}, D-pClPhe^{2}, D-3-Pal^{3}, PicLys^{5}, D-PicLys^{6}, IOrn^{8} and D-Ala^{10};

N-Ac-D-2-Nal^{1}, D-pClPhe^{2}, D-3-Pal^{3}, MNicLys^{5}, D-MNicLys^{6}, IOrn^{8} and D-Ala^{10};

N-Ac-D-2-Nal^{1}, D-pClPhe^{2}, D-3-Pal^{3}, PzcLys^{5}, D-PzcLys^{6}, IOrn^{8} and D-Ala^{10};

N-Ac-D-pClPhe^{1}, D-3-Pal^{3}, Tyr^{5}, D-NicLys^{6} and ILys^{8};

N-Ac-D-Cl_2Phe^{1}, D-3-Pal^{3}, Tyr^{5}, D-NicLys^{6} and ILys^{8};

acylated Lys^{5}, D-acylated Lys^{6} and N-alkylated diamino acid^{8};

NicLys^{5}, D-NicLys^{6} and ILys^{8};

PicLys^{5}, D-PicLys^{6} and ILys^{8};

NicLys^{5}, D-NicLys^{6} and IOrn^{8};

PicLys^{5}, D-PicLys^{6} and IOrn^{8};

MNicLys^{5}, D-MNicLys^{6} and IOrn^{8};

PzcLys^{5}, D-PzcLys^{6} and IOrn^{8};

Tyr^{5}, D-NicLys^{6} and ILys^{8};

Tyr^{5}, D-NicLys^{6} and IOrn^{8};

N-Ac-D-2-Nal^{1}, D-pClPhe^{2}, D-3-Pal^{3}, Ser^{4}, NicLys^{5}, D-NicLys^{6}, Leu^{7}, ILys^{8}, Pro^{9} and D-Ala$^{10}NH_2$; and

N-Ac-D-2-Nal^{1}, D-pClPhe^{2}, D-3-Pal^{3}, Ser^{4}, PicLys^{5}, cis D-PzACAla^{6}, Leu^{7}, ILys^{8}, Pro^{9} and D-Ala$^{10}NH_2$.

The present invention further involves use of the above decapeptides in a process for inhibiting ovulation in an animal. This process comprises administering to said animal a decapeptide preferably having the structure: N-Ac-D-2-Nal^{1}, D-pClPhe^{2}, D-3-Pal^{3}, Ser^{4}, NicLys^{5}, D-NicLys^{6}, Leu^{7}. ILys^{8}, Pro^{9} and D-Ala$^{10}NH_2$. Likewise, the inventive process may be used to inhibit ovulation in an

animal; to inhibit the onset of puberty in an animal; to inhibit the sexual impetus of an animal; to alter the gonadal function of an animal; to inhibit the growth of hormone-dependent tumors in an animal; and to lower LH and FSH levels in serum of post-menopausal women. These and other related uses will be apparent to those skilled in the art upon examination of this specification.

Abbreviations and formulas used herein include the following:

a	=	alpha
BOC	=	t-butoxycarbonyl
Br-Z	=	o-bromobenzyloxycarbonyl
nBuOAc	=	n-butylacetate
n-BuOH	=	n-butanol
c	=	cis
$CDCl_3$	=	deuterochloroform
$CHCl_3$	=	chloroform
CH_2Cl_2	=	dichloromethane
CH_3CN	=	acetonitril
Cl-Z	=	o-chlorobenzyloxycarbonyl
d	=	delta
DCC	=	dicyclohexylcarbodiimide
DIEA	=	diisopropylethylamine
DMF	=	dimethylformamide
E	=	eta
Et	=	ethyl
EtOAc	=	ethyl acetate
EtOH	=	ethanol
Et_2O	=	diethyl ether
HF	=	hydrogen fluoride
HOAc	=	acetic acid
KH_2PO_4	=	potassium dihydrogen phosphate
MeOH	=	methanol
$MgSO_4$	=	magnesium sulfate

NH_4OAc	=	ammonium acetate
iPrOH	=	2-propanol
py	=	pyridine
t	=	<u>trans</u>
TFA	=	trifluoroacetic acid
THF	=	tetrahydrofuran
TOS	=	p-toluensulfonyl
m	=	micro
Z	=	benzyloxycarbonyl
Abu	=	2-aminobutyric acid
Aile	=	alloisoleucine
AnGlu	=	4-(4-methoxyphenylcarbamoyl)-2-aminobutyric acid
BzLys	=	N^E-benzoyllysine
Cit	=	citrulline
Cl_2Phe	=	3,4-dichlorophenylalanine
CypLys	=	N^E-cyclopentyllysine
DMGLys	=	N^E-N,N-dimethylglycyl)lysine
Dpo	=	N^d-(4,6-dimethyl-2-pyrimidyl) ornithine
Et_2hArg	=	N^G,N^G-diethylhomoarginine
FPhe	=	A-fluorophenylalarine
HOBLys	=	N^E-(4-hydroxybenzoyl)lysine
Ilys	=	N^E-isopropyllysine
INicLys	=	N^E-isonicotinoyllysine
IOrn	=	N^d-isopropylornithine
Me_3Arg	=	N^G,N^G,N^{G1}-trimethylarginine
Me_2Lys	=	N^E,N^E-dimethyllysine
MNicLys	=	N^E-(6-methylnicotinoyl)lysine
MPicLys	=	N^E-(6-methylpicolinoyl)lysine
NACAla	=	3(4-nicotinoylaminocyclohexyl)alanine
2-Nal	=	3-(2-naphthyl)alanine
NicLys	=	N^E-nicotinoyllysine
NicOrn	=	N^d-nicotinoylornithine
Nle	=	norleucine, 2-aminohexanoic acid

NMeLeu	=	N-methylleucine
Nval	=	norvaline, 2-aminopentanoic acid
3-Pal	=	3-(3-pyridyl)alanine
pClPhe	=	3-(4-chloro)phenylalanine
PicLys	=	N^E-picoloyllysine
Pip	=	piperidine-2-carboxylic acid
PmcLys	=	N^E-(4-pyrimidinylcarbonyl)lysine
PmACAla	=	3[4(4-pyrimidinylcarbonyl)aminocyclohexyl]alanine
PzACAla	=	3(4-pyrazinylcarbonylaminocyclohexyl)alanine
3-PzAla	=	3-pyrazinylalanine
PzcLys	=	N^E-pyrazinylcarbonyllysine
Sar	=	N-methylglycine
TinGly	=	3-thienylglycine

Most natural amino acids were obtained from Peninsula Laboratories, San Carlos, CA. The hydroxyl group of Ser was protected as the benzyl ether, the phenolic hydroxyl group of Tyr as the Br-Z derivative, and E-amino group of Lys as the Cl-Z derivative, the guanidino group of Arg and the imidazole group of His as the TOS derivatives. The a-amino function was protected as the BOC derivative. BOC-Orn(Z) was obtained from Sigma Chemical Co., St. Louis, Mo. BOC-D-2-Nal, BOC-D-3-Pal, BOC-D-Cl_2Phe, BOC-pClPhe and BOC-ILys(Z) dicyclohexylamine salt were provided by the Southwest Foundation for Biomedical Research, San Antonio, TX. The benzhydrylamine hydrochloride resin was obtained from Beckman Bioproducts, Palo Alto, CA. The nitrogen content was about 0.65 mmoles/g. The CH_2Cl_2 was distilled before use.

The present invention involves the design, synthesis and use of LHRH antagonists with high antiovulatory potency and diminished activity to release histamine (1).

These new antagonists feature, for example, D-N^E-nicotinoyllsine (D-NicLys) in position 6 and N^E-isopropyllysine (ILys) in position 8. The solution of D-Arg^6, particularly in combination with Arg^8 and a cluster of hydrophobic aromatic amino acid residues at the N-terminal, have been implicated in the release of histamine (2-4).

Other reductions of anaphylactoid activity were obtained by increasing the distance between the positive charges in positions 6 and 8 by Arg^5 and by inclusion of a neutral residue in position 6 as in [N-Ac-D-2-Nal^1,D-$pClPhe^2$,D-3-Pal^3,Arg^5,D-4(p-methoxybenzoyl)-2-amino-butyric $acid^6$,D-Ala^{10}]-LHRH (2-Nal represents 3-(2-naphthyl) alanine; PClPhe represents 3(4-chlorophenyl)alanine; 3-Pal represents 3(3-pyridyl)alanine) by Rivier <u>et al.</u> (5) and [N-Ac-D-2-Nal^1,D-$aMepClPhe^2$,D-Trp^3,Arg^5,D-Tyr^6,D-Ala^{10}]-LHRH (aMepClPhe represents 2 methyl-3(4-chlorophenyl)alanine) by Roeske <u>et al.</u> (6). Further modifications in position 6 are reductive alkylation of D-Lys^6 by Hocart <u>et al.</u> (7), incorporation of N,N-diethylhomoarginine by Nestor <u>et al.</u> (9). The cyclic analogs recently synthesized by Rivier <u>et al.</u> did not show any lowering in histamine release compared to the linear counterparts (10).

From the peptides of the present invention, two were initially selected as models for further design. The peptide [N-Ac-D-2-Nal^1, D-$pClPhe^2$, D-3-Pal^3, $NicLys^5$, D-$NicLys^6$, $ILys^8$, D-Ala^{10}]-LHRH (named Antide) had an impressive combination of potency and low histamine release; antiovulatory activity (AOA) was 100% at lug and 36% at 0.5ug; ED_{50} for histamine release, <u>in vitro</u>, was consistently above 300ug/ul as compared to about 0.17 for the standard analog [N-Ac-D-2-Nal^1,D-$pFPhe^2$,D-Trp^3,D-Arg^6]-LHRH (pFPhe represents 3(4-fluorophenyl)alanine)

(5). Another analog was identical to Antide except for $PicLys^5$ and $D\text{-}PicLys^6$ (PicLys represents N-picoloyllysine); 100% AOA at 0.5ug and 40% at 0.25ug; ED_{50}, 93±11.

Included herein are results from LHRH analogs with acylated aminocyclohexylalanine residues in position 6, from analogs in which Leu^7 has been substituted with other neutral residues, from a comparison of $ILys^8$ vs. $IOrn^8$, and from tests on oral activity and duration of antagonists activity when administered orally or parenterally (s.c.)

Melting points are uncorrected. NMR data are reported as d-values downfield from TMS.

Before acylation, the Z and Cl-Z groups of Lys and Orn were cleaved by hydrogenolysis in MeOH in the presence of 10% Pd/C.

<u>BOC-D-BzLys</u> was synthesized by acylation of BOC-D-Lys with benzoyl chloride as described for the L- isomer by Bernardi <u>et al</u>. (17).

<u>BOC-DMG-Lys</u> was prepared by acylation of BOC-Lys with chloracetyl chloride using the same method and the reacting the crude product from 10 mmoles BOC-Lys in 10 ul THF with 10 ul 40% aq. dimethylamine. The reaction mixture was stirred 15 minutes in ice bath and then 2.5 hours at room temperature. After evaporation in vacuo the crude product was dissolved in 10 ul H_2O and applied on a Bio-Rad AG1-X8 column, acetate form, 1 x 25 cm. The column was first washed with 200 ul water and then the product was eluted with 6% HOAc and lyophilized several times to remove the HOAc. Yield 60-70%. Amorphous mass. R_f (n-BuOH:py:HOAc:H_2O = 30:10:3:12) = 0.27. Purity >

95%. NMR ($CDCl_3$):1.45,s,9H,t-butoxy group; 1.85-1.48,m,6H,B,y,d,CH_2 groups; 2.6,s,6H,$N(CH_3)_2$; 3.25,m,2H, E-CH_2; 3.37,s,2H,N-CH_2-CO; 4.15,m,1H,a-CH.

The other acylated Lys derivatives in the tables were prepared from BOC-D or L-Lys and the corresponding p-nitrophenyl ester.

<u>p-Nitrophenyl nicotinate</u>. To 9.85 g, 80 mmoles, nicotinic acid and 13.35 g, 96 mmoles p-nitrophenol in 250 ul DMF was added 16.5 g, 80 mmoles DCC with stirring in ice-bath. After 1 hour at O`C and 3 hours at room temperature the urea was filtered off and the product was precipitated by the addition of an equal volume of water. Filtration, drying in vacuo and recrystallization from i-PrOH gave 11.22 g, 57% of white needles, m.p. 172.5-173`C (24)

<u>p-nitrophenyl isonicotinate</u> was prepared, in the same manner 12 g, 61%, m.p. 139-141`C, m.p. 137-139`C. (18)

Also p-nitrophenyl 6-methylnicotinate was prepared in the same way. Yield from 70 mmoles 6-methylnicotinic acid: 6.0 g, 33% after recrystallization from MeOH. M.p. 156-157`C. R_f (2% MeOH in $CHCl_3$) = 0.57 NMR ($CDCl_3$): 2.7,s,3H,CH_3; 7.36,d,1H,py H^5;7.45,m,2H,H adjacent to the oxygen in the phenyl ring; 8.34,m,3H,H adjacent to the NO_2 group in the phenyl ring overlapping with py H^4; 9.27,d,1H,py H^2.

<u>P-nitrophenyl picolinate</u>. 4.92 g, 40 mmoles, picolinic acid and 5.84 g, 42 mmoles p-nitrophenol were suspended/dissolved in 200 ul CH_2Cl_2. Then 8.24 g 40 mmoles, DCC was added in 20 ul CH_2Cl_2 with vigorous stirring. Stirring was continued in room temperature for 17 hours. Then the mixture was filtered and the filter

cake washed with 30-40 ul CH_2Cl_2. The raw product was first treated with 100 ul Et_20 with stirring in ice-bath and filtered. Recrystallization from 250 ul iPrOH gave 6.24 g, 63% product. M.p. 154-6`C (dec.). M.p. 145-7`C (18).

Pyrazinecarboxylic acid p-nitrophenylester. This compound was prepared using the same method as the previous compound. From 40 mmoles pyrazinecarboxylic acid and 44 mmoles p-nitrophenol was obtained 35.2 mmoles, 88%, ester. M.p. 180-182`C (dec.). R_f ($CHCl_3$:MeOH = 49:1) = 0.72. NMR ($CDCl_3$): 7.5,m and 8.37m,2H each, hydrogens adjacent to the oxygen and nitro group respectively in the phenol ring; 8.84,m,1H,pyrazine H^5; 8.9,d,1H,pyrazine H^6; 9.48,d,1H,pyrazine H^3.

BOC-NicLys. 2.5 g BOC-Lys (L or D) was suspended in 200 ul DMF with stirring. Then 1.1 equivalent of p-nitrophenyl nicotinate was added and the mixture stirred at room temperature for 36 hours. The mixture was then filtered and the filtrate evaporated to dryness at reduced pressure to yield a yellow oil. The residue was stirred with 2x50 ul Et_20 in ice-bath. The first Et_20 phase was decanted, the second was filtered off. Recrystallization from EtOAc/hexanes gave 2.05 g product, 58% (L-form). M.p. 138`C, lit. (17) 138-141`C. L-form $[a]^{20}_D$= -2.91` (MeOH), D-form $[a]^{20}_D$= 3.35` (MeOH).

L- and D-BOC-INicLys were prepared similarly by acylating 10 mmoles L or D BOC-Lys with p-nitrophenyl isonicotinate in 100 ul DMF, 40 hours, room temperature. The crude product was partitioned between 120 ul EtOAc and 50 ul H_2O. The EtOAc phase was extracted with 2 x 50 ul H_2O and 50 ul brine. The original aqueous phase was back-extracted with 30 ul EtOAc. The combined EtOAc phases were then dried ($MgSO_4$) and evaporated and the

residue was treated with Et_2O and recrystallized as above to give 1.07 g, BOC- L-INicLys, 30.5%. The yield for the D compound was 1.26 g, 36%.. NMR (Acetone d_6): 1.4,s,9H,t-butoxy group; 1.8-1.48,m,6H,B,y,d,$-CH_2-$; 3.44,t,2H,E-CH_2; 4.13,m,1H,a-CH; 7.77,m,2H,py H^5 and H^3; 8.70,m,2H,py H^2 and H^6.

<u>L- and D-BOC-PicLys</u>. 1.23 g, 5 mmoles, of L- or D-BOC-Lys was stirred with 1.34 g, 5.5 mmoles, p-nitrophenyl picolinate in 60 ul DMF for 16 hours. After filtration and evaporation and product was purified by column chromatography on silica gel on a 4.5 x 32 cm column and the solvent system n-BuOH:py:HOAc:H_2O = 30:10:3:12. The product after chromatography was dissolved in EtOAc and washed with H_2O, brine, dried and evaporated in vacuo. The yields were usually 60-70%. NMR ($CDCl_3$): 1.43,s,9H,t-butoxy group; 1.73-1.45,m,6H,B,y,d-CH_2; 3.47,m,2H,E-CH_2; 4.32,m,1H,a-CH; 7.43,m,1H,py H^5; 7.85,m,1H,py H^4; 8.2,m,1H,py H^3; 8.55,m,1H,py H^6.

<u>L- and D-BOC-MNicLys</u>. 10 mmoles BOC-Lys and 10.5 mmoles p-nitrophenyl 6-methylnicotinate were allowed to react in 150 ul DMF in the usual manner. After 27 hours filtration and evaporation yielded a yellow oil. Et_2O treatment (2 x 50 ul) gave 3.3 g product which was recrystallized from 50 ul 20% MeOH in EtOAc/hexane. Yield 2.87 g, 78.6% (L-form). R_f(n-BuOH:py:HOAc:H_2O = 32:10:3:12) = 0.61. NMR($CDCl_3$): 1.46,s,9H,t-butoxy group; 1.9-1.5,m,6H,B,y,d-CH_2; 2.57,s,3H,py CH_3; 3.36,m,2H,E-CH_2; 4.11,m,1H,a-CH; 7.22,d,1H,py H^5; 8.08,m,1H,py H^4; 8.95,broad s,1H,py H^2.

<u>L- and D-BOC-PzcLys</u>. Using the method above was obtained from 7.7 mmoles pyrazine carboxylic acid p-nitrophenyl ester and 7 mmoles BOC-Lys, L or D, in 100 ul DMF about 6 mmoles product after recrystallization from

iPrOH. R_f(n-BuOH:py:HOAc:H_2O = 30:10:3:12) = 0.47. NMR ($CDCl_3$): 1.45,s,9H,t-butoxy group; 1.9-1.48,m,6H,B,y,d-CH_2-; 3.51,m,2H,E-CH_2; 4.29,m,1H,a-CH; 8.52,q,1H,pyrazine H^5; 8.77,d,1H,pyrazine H^6; 9.41,d,1H,pyrazine H^3.

BOC-L-NicOrn. This compound was prepared the usual way by reacting 7 mmoles p-nitrophenyl nicotinate with 5 mmoles BOC-Orn in 75 ul DMF for 36 hours. Evaporation and recrystallization from EtOAc gave 3.5 mmoles, 70%, NicOrn, m.p. 143-144`C. R_f(n-BuOH:HOAc:H_2O = 4:1:2) = 0.70. NMR($CDCl_3$): 1.45,s,9H,t-butoxy group; 7.46,m,1H,py H^5; 8.27,m,1H,py H^4; 8.69,m,1H,py H^6; 9.05,m,1H,py H^2.

BOC-D-trans-NACAla. 1.43 g, 5 mmoles, BOC-D-trans-3(4-aminocyclohexyl) alanine (provided by the Southwest Foundation for Biomedical Research) was stirred with 1.35 g, 5.5 mmoles, p-nitrophenyl nicotinate in 60 ul DMF for 120 hours in room temperature. The mixture was then filtered, evaporated, treated with Et_2O in ice bath and filtered again. Recrystallization was done by heating in 12 ul EtOH and adding 18 ul hot H_2O. This produced a clear solution from which crystals separated on cooling. This procedure was repeated twice. Yield: 0.98 g, 50%. Purity >95%. M.p. >220`C. NMR(DMSO d_6): 1.46,s,9H,t-butoxy group; 1.9-1.48,m,11H,ring CH_2, ring CH in position 1 and B-CH_2; 3.72,m,1H,ring CH in position 4; 3.95,m,1H, a-CH; 7.48,m,1H,py H^5; 8.16,m,1H,py H^4; 8.67,m,1H,py H^6; 8.96,m,1H,py H^2.

BOC-D-cis-NACAla. 5 mmoles BOC-D-cis-3(4-aminocyclohexyl)alanine (source: as above) and 5.5 mmoles p-nitrophenyl nicotinate were allowed to react in DMF as above. Reaction time: 25 hours. Purification was achieved by Et_2O treatment as above and silica gel chromatography on a 4.5 x 32 cm column using the solvent system $CHCl_3$:MeOH:py:HOAc = 75:10:10:5. Yield 1.3 g, 61%,

amorphous powder. R_f (column system) = 0.58. NMR ($CDCl_3$): 1.44,s,9H,t-butoxy group; 1.95-1.45,m,11H,ring CH_2, ring CH in position 1 and B-CH_2; 4.22,m,1H,a-CH; 4.35,m,1H,ring CH in position 4: 7.35, 8.24, 8.63 and 8.98, 1H each, assignments as previous compound.

<u>BOC-IOrn(Z)</u>. This compound was prepared from BOC-Orn(Z) by reductive alkylation with acetone and H_2/Pd as described by Prasad <u>et</u> <u>al</u>. (23) followed by conversion to the Nd- Z derivative with benzyl chloroformate in aqueous alkali (Schotten-Baumann conditions). Purification was achieved by chromatography on silica gel with $CHCl_3$/MeOH 85:15. R_f ($CHCl_3$;MeOH:HOAc = 85:15:3) = 0.8. NMR($CHCl_3$): 1.10,d,6H, isopropyl CH_3; 1.40,s,9H,t-butoxy group; 1.7-1.5,m,4H,B,y-CH_2; 3.09,m,2H, d-CH_2; 4.2,m,1H,a-CH; 5.10,s,2H,benzyl CH_2; 7.3,m,5H,aromatics.

<u>BOC-CypLys(Z)</u>. 2.04 g BOC-Lys(Z) was dissolved in 8 ul of cyclopentanone and 32 ul H_2O containing 0.22 g NaOH. Hydrogenation was performed in the presence of 0.4 g 10% Pd/C at 50-60 psi in a Parr apparatus. After 4 hours the hydrogenation was interrupted and 2 ul 0.5 M NaOH and 10 ul MeOH were added. The hydrogenation was then continued for 16 hours at 50-60 psi. Then filtration and evaporation. The residue was dissolved in 75 ul H_2O and the aqueous phase extracted with three times with Et_2O and once with hexane. The pH was then brought to 6-7 with HCl and the solution evaporated in rotary evaporator, bath temperature 40`C. The resulting product was then converted to the Z-derivative using benzyl chloroformate in aqueous NaOH (Schotten-Baumann conditions). Yield: 1.3 g, 58% overall. R_f (n-BuOH:py:HOAc:H_2O - 30:10:3:12) = 0.69. Purity >95%. NMR ($CDCl_3$): 1.45,s,9H,t-butoxy group; 1.95-1.35,m,14H,ring CH_2 + B,y,d-CH_2; 3.13,broad t,2H,E-CH_2; 4.34-4.05,m,2H,a-CH + ring CH; 5.13,s,2H,benzyl CH_2; 7.35,m,5H,aromatic protons.

BOC-Me_2Lys, D- and L-. These compounds were prepared by hydrogenolysis of the corresponding Z- or Cl-Z-derivatives in the presence of 37% formaldehyde essentially as described by L. Benoiton (22) for the N^a -acetyl analog. Purification was achieved by chromatography on silica gel with the solvent system n-BuOH:py:H_2O = 2:2:1. The yields are 40-65% and the products are amorphous. NMR ($CDCl_3$): 1.41,s,9H,t-butoxy group; 1.9-1.5,m,6H,B,y,d-CH_2; 2.6,s,6H,$N(CH_3)_2$; 2.8,m,2H,E-CH_2; 4.03,m,1H,a-CH.

BOC-D-AnGlu. 0.62 g, 3 mmoles, DCC was added to the ice-cooled solution of 1.10 g, 3 mmoles, BOC-D-glutamic acid a-benzylester and 0.39 g, 3 mmoles, p-anisidine in 25 ul CH_2Cl_2. The reaction mixture was stirred while warming up to room temperature and then another 17 hours. The dicyclohexylurea was then filtered off and $CHCl_3$ added to a total volume of 125 ul. This solution was extracted with 2 x 1N H_2SO_4, H_2O, saturated $NaHCO_3$, 2 x H_2O and dried ($MgSO_4$). Evaporation and recrystallization from EtOH gave 0.99 g, 74% product, m.p. 129.5-131`C. R_f (4% MeOH in $CHCl_3$) = 0.53. This product was dissolved in 30 ul MeOH and 10 ul EtOH and hydrogenated in the presence of 0.3 g Pd/C at 50 psi for 2.5 hours. Filtration and evaporation gave a quantitative yield of BOC-D-AnGlu. Not crystalline. Purity >98%. NMR ($CDCl_3$): 1.45,s,9H,t-butoxy group; 2.35-1.95,m,2H,B-CH_2; 2.6-2.4,m,2H,y-CH_2; 3.76,s,3H,OCH_3; 4.3,m,1H,a-CH; 6.82 and 7.42, broad d, 2H each, aromatic protons.

BOC-Me_3Arg. First, N,N,N',S-tetramethylisothiourea was prepared by the procedure of Lecher and Hardy (19). B.p. (15 mm) = 74`C, lit(above) 68`C at 11 mm. BOC-Orn,9 mmoles, and teramethylisothiourea, 10 mmoles, were dissolved in 15 ul DMF and 2 ul triethylamine and incubated at 100`C for 2 hours and at room temperature for

10 hours. Then the reaction mixture was evaporated to dryness and passed through a silica gel column eluted by iPrOH:triethylamine:H_2O = 42:6:13. The white solid so obtained was dissolved in H_2O and the solution was acidified with 6N HCl and lyophilized to give 5.5 mmoles product. R_f (column eluant) = 0.50. NMR (D_2O): 1.42,s,9H,t-butoxy group, 2.80,m,1H,a-CH; 2.89,s,3H, CH_3 on guanidino group; 2.96,s,6H, $(CH_3)_2N$; 3.25,t,2H,d-CH_2; 1.50,m,4H,B,y-CH_2.

BOC-Dpo. From 10 mmoles arginine hydrochloride and 1.72 g sodium hydrogen carbonate dissolved in 17 ul H_2O, 28.6 ul acetylacetone and 28.6 ul EtOH was obtained 7.5 mmoles Dpo following the procedure of F.-S. (20). The product was then converted to the corresponding BOC-derivative using di-t-butyl dicarbonate in 50% aqueous dioxane in the presence of sodium hydroxide. This reaction proceeds in essentially quantitative yield. R_f(nBuOH:HOAc:H_2O = 4:1:2) = 0.63. NMR ($CDCl_3$): 1.45,s,9H,t-butoxy group; 1.9-1.5,4H,B,y-CH_2; 2.33,s,6H,CH_3; 3.46,m,2H,d-CH_2; 4.24,m,1H,a-CH; 6.35,s,1H, aromatic H. L- and D- forms react similarly.

BOC-D-Et_2hArg. This compound was prepared by the method of Nestor and Vickery, U.S. Pat. 4,530,920, July 23, 1985. R_f(nBuOH:HOAc:H_2O = 4:1:2) = 0.52.

The peptides of the present invention were synthesized by the solid phase method using a Beckman Model 990 Peptide Synthesizer. (1, 11) The benzhydrylamine hydrochloride resin (BHA-resin) was used as a solid support. The program of the synthesizer was divided into subprograms.

1. Deprotection: 1. CH_2Cl_2 (2 x wash, 1 or 2 min); 2. 50% TFA in CH_2Cl_2 containing 0.1% indole (1 x

wash, 1 or 2 min); 3. 50% TFA in CH_2Cl_2 containing 0.1% indole (deprotection, 20 min); 4. CH_2Cl_2 (2 x wash).

2. Neutralization: 1. CH_2Cl_2 (2 x wash, 1 or 2 min); 2. DIEA (10% in CH_2Cl_2) (2 x wash, 1 or 2 min); 3. DIEA (10% in CH_2Cl_2) (neutralization, 5 min); 4. CH_2Cl_2 (2 x wash, 1 or 2 min).

3. DCC Coupling: 1. CH_2Cl_2 (2 x wash, 1 or 2 min); 2. amino acid solution in CH_2Cl_2 (delivery, transfer, mix, 5 min); 3. DCC (10% in CH_2Cl_2, (delivery and mix, 180 min); 4. CH_2Cl_2 (2 x wash, 1 or 2 min).

4. Active Ester Coupling: not used.

5. Final Wash: 1. CH_2Cl_2 (2 x wash, 1 or 2 min); 2. i-PrOH (3 x wash, 1 or 2 min); 3. DMF (3 x wash, 1 or 2 min); 4. CH_2Cl_2 (3 x wash, 1 or 2 min).

6. Wash after TFA Treatment: 1. CH_2Cl_2 (2 x wash, 1 or 2 min); 2. i-PrOH (2 x wash, 1 or 2 min); CH_2Cl_2 (3 x wash, 1 or 2 min).

7. Acetylation: 1. CH_2Cl_2 (2 x wash, 1 or 2 min); 2. 25% Ac_2O and Py in CH_2Cl_2 (1 x wash, 1 or 2 min); 3. 25% Ac_2O and Py in CH_2Cl_2 (acetylation, 20 min); 4. CH_2Cl_2 (2 x wash, 1 or 2 min).

The first amino acid was attached to the resin by the program sequence 2-3-5. Before placing the resin into the reaction vessel, the resin was washed in a separatory funnel with 25 ul CH_2Cl_2/g resin to remove the fine particles. In all couplings, usually a 3-4 fold excess of the Boc-amino acid over the nitrogen content of the resin was used. This procedure generally resulted in a complete coupling reaction. If a positive ninhydrin color reaction

was observed, a second coupling was performed (program sequence 3-5). Then, the resin was acetylated (program sequence 7-5).

The next amino acid was attached by the program sequence 1-6-2-3-5. For DCC coupling, all amino acids were dissolved in CH_2Cl_2. Acetylation of the amino acid residue in position 1 was performed using the program sequence 1-6-2-7-5. The volume of the solvents and the reagents used for the washing and the performing of the chemical reactions was about 10 ul/g resin.

After all of the amino acids had been coupled, the peptide resin was dried overnight, in vacuo. The resin was then treated with double-distilled liquid hydrogen fluoride (10 ul/g resin) containing 10-25% distilled anisole or p-cresol for 1 hour at 0°C. Then, the HF was evaporated under reduced pressure and the residue was dried overnight, in vacuo, by an oil pump. The mixture was then extracted several times with Et_2O (25 ul/g resin), then with aqueous. HOAc, 30%, 50%, 10%, and once with 25 ul distilled, deionized water. The combined aqueous solution was lyophilized to yield the crude peptide.

Most peptides were purified by silica gel chromatography (1 x 60 cm column) using one of the solvent systems $nBuOH:HOAc:H_2O$ = 4:1:2 or 4:1:5 upper phase or $nBuOAc:nBuOH:HOAc:H_2O$ = 2:8:2:3 followed by gel filtration over Sephadex G 25 with 6% HOAc as the eluant. In the case of unsatisfactory purity after this procedure the peptides were further purified by semipreparative HPLC using a Waters liquid chromatograph equipped with a 660 solvent programmer. A 1.2 x 25 cm m-Bondapak C_{18} column was used with the solvent system A = 0.1 M NH_4OAc pH 5.0 and B = 20% A + 80% CH_3CN. Different gradients of

increasing amounts of B in 15 - 25 minutes were employed to effect purification.

An alternate purification scheme has been gel filtration over Sephadex G-25 with 6% HOAc followed by chromatography over Sephadex LH 20 (2.5 x 100 cm) with the solvent system H_2O:nBuOH:HOAc:MeOH = 90:10:10:8. If necessary, the latter procedure was repeated 1 - 2 times.

The purity of the peptides was assessed by thin layer chromatography on Merck silica gel plates in at least four different solvent systems as shown in Table II. The spots were developed with the chlorine/o-tolidine reagent. In Table II are also shown the conditions and results of analytical HPLC. The equipment was the one described above except that an analytical m-Bondapak C_{18} column (3.9 mm x 30 cm) was used.

Amino acid analyses were performed on a Beckman model 118 CL amino acid analyzer. Samples of about 0.5 ug were hydrolyzed in 6N hydrochloric acid in sealed glass tubes for 24 hours at 110`C. The residue was then evaporated and dissolved in citrate buffer, pH 2.2 and applied to the analyzer. The results are in Table III.

The antiovulatory activity, AOA, in rats was determined as described by Humphries et al. (12). The wheal test was performed by intradermally injecting 10 ug of peptide in 100 ul of saline into anaesthesized rats, measuring the ideally circular wheal response and calculating the area. The *in vitro* histamine release test was done as described by Karten et al. (4).

The results of these bioassays are presented in Table I and other Tables appended hereto.

Of the 57 peptides in Table I, 21 had an AOA of about 90% or more at a dosage of 1 ug in the present assay. Of the 37 peptides of Table 1 tested for histamine release in the rat mast cell assay, 10 had E_D50 values of 300 or more as compared to 0.17 for the standard compound [N-Ac-D-2-Nal^1,D-4-F-Phe^2,D-Trp^3,D-Arg^6]-LHRH. Nine additional analogs had E_D50 values ranging from 86 to 288, i.e. they do not release more histamine than clinically used "superagonists".

Of the thirty-seven peptides of Table 1 tested in the rat mast cell assay, seven (numbers 4, 23, 24, 43 (Antide), 44, 53, 55) had <u>both</u> an AOA of about 90% or more at 1 ug <u>and</u> an E_D50 value of about $\geq$ 86 ug/ul. This included the potent analog, No. 53, which had 100% AOA at 0.5 ug and 40% AOA at 0.25 ug. The E_D50 value for this analog was 93$\pm$28. It was thus demonstrated that high AOA with low histamine release could be found in the analogs of the present invention.

Structural features in common for these seven peptides are: 1) A D-Lys residue in position 6 which was acylated by the weakly basic nicotinic acid or analogs like picolinic and 6-methylnicotinic acid. 2) The corresponding acylated L-Lys residue or the natural Tyr in position 5. 3) The alkylated derivatives ILys or IOrn in position 8. 4) Arg is absent from the sequence.

Two examples of the influence of Arg on histamine release are the pairs 43,10 and 4,1. No. 43 (Antide) has the sequence N-Ac-D-2-Na^1,D-$pClPhe^2$sub,D-3-Pal^3,Ser^4,$NicLys^5$,D-$NicLys^6$,Leu^7,$ILys^8$,Pro^9,D-Ala^{10}-NH_2. Its E_D50 value is >300. No. 10 is identical in sequence except that $NicLys^5$ is replaced by Arg^5. This caused the E_D50 value to decrease to 4.3$\pm$0.52. No. 4 has identical sequence as No. 43 except for Tyr in position 5. Its E_D50

value is 133$\pm$22. In No. 1, ILys^8 in this sequence is replaced by Arg^8 which caused the E_D50 value to decrease to 39.2$\pm$7. It thus seems that position 5 is more sensitive than position 8 for Arg substitution.

In position 8, the alkylated ILys and IOrn residues are superior to Lys and Orn, respectively, both with respect to AOA and histamine release (pairs 3,4 and 6,7). Whether ILYs^8 or IOrn^8 is best seems to be sequence dependent.

For the determination of duration of action, the antagonist was administered s.c. or orally to 26 days old female rats at a specific time before administration of the agonist, [D-Qal^6]-LHRH. The serum levels of rat luteinizing hormone (LH) and rat follicle stimulating hormone (FSH) were then measured 2 hours after the agonist administration by RIA. The oral administration was done through force-feeding with feeding tubes.

Table IV shows data on AOA and histamine release for analogs containing acylated aminocyclohexylalanine residues. For the analogs with NicLys^5, D-NACAla^6, IV-1 and IV-2, (NACAla represents 3(4-nicotinoyl-aminocyclohexyl)alanine), analog 2 with cis-D-NACAla^6 is somewhat more active, 100% vs. 70% AOA at lug. Analogs IV-7 and IV-8 with NicLys^5, D-PzACAla^6 (PzACAla represents 3(4-pyrazinylcarbonylaminocyclohexyl)alanine) show the opposite order of activity. The trans residue has the higher AOA, 88% vs. 25% at lug.

Analogs IV-3 and IV-4 with PicLys^5, trans and cis PACAla^6 (PACAla represents 3(4-picolinoylaminocyclohexyl)alanine) are equipotent, 50 and 54% AOA at 0.5ug, respectively, whereas in the case of PicLys^5, trans and cis PzACAla^6 the cis compound is more

than twice as active. The former, analog IV-5 is about as potent as analogs IV-3 and IV-4 (44% at 0.5ug) while the latter, analog 6, has 100%, 73%, and 29% AOA at 0.5, 0.25, and 0.125ug, respectively. The high potency analog IV-6 is unique in comparison with the low activity of the structurally similar analog IV-8.

Analog IV-9 has cis-PzACAla^5, D-PicLys^6 and, although residues 5 and 6 are reversed, retained the high potency of analog IV-6, 90% and 67% at 0.5 and 0.25ug, respectively.

As for histamine release, all analogs tested, *in vitro*, have lower ED_{50} values than the parent compounds. The ED_{50} values range from about 30 to about 60 compared to >300 and 93±11 for Antide and analog V-10. The tests for wheal response show a range from 99.5 to 129.6, which is similar to Antide (132.7) and analog V-10 (123.0). The lack of correlation between the two tests may primarily reflect assay variation.

In summary, for the analogs with NicLys^5, incorporation of aminocyclohexylalanine derivatives in position 6 resulted in substantial increase in, *in vitro*, histamine release and unchanged or lowered AOA. For the PicLys^5 analogs with the same substitutions there was lowering of AOA potency in all cases except one, where a considerable increase was observed. The combination PicLys^5 and cis-D-PzACAla^6 evidently possesses some beneficial structure. Histamine release for the PicLys^5 analogs was increased by 50-100%.

In Table V, are the results from substitutions in position 7 of analog V-10. This position allows some structural freedom although none of the peptides show higher AOA than analog V-10. Analogs V-12, V-14, and V-16

having $Aile^7$ (alloisoleucine), Val^7 and Abu^7 (2-aminobutyric acid), are equipotent with analog V-10. Analog V-16 with the straight chain Abu^7 is slightly more potent than analogs V-13 and V-15 with Nle^7 (norleucine) and $Nval^7$ (norvaline), respectively, which should more closely resemble the natural Leu^7.

For compound V-17 with the small Ala^7, the AOA decreased to 60% at 0.5ug. Incorporation of Trp^7 which is the natural residue in chicken II, salmon and lamprey LHRH's (13-15), gave analog 18 with only 10% AOA at 0.5ug. Trp^7 may be too large considering the size of the adjacent D-$PicLys^6$ and $Ilys^8$.

The most interesting feature of Table V is the, *in vitro*, histamine release data. The three analogs with similar AOA potency as analog V-10 show markedly diminished histamine release. The ED_{50} values for analogs V-12, V-14, and V-16 are >300, 213±30 and 273±27, respectively; i.e., a 2-3 fold decrease in histamine release is achieved by small changes in side chain structure. Also, the wheal response is diminished for all analogs compared to V-10.

It was noted earlier (1) that whether ILys or IOrn is the best substituent in position 8 is sequence dependant. To further investigate this aspect, the $IOrn^8$ analogs corresponding to some of the best peptides were synthesized and tested. The results in Table VI indicate that $ILys^8$ may be better. For two of the pairs, analogs VI-10, VI-19 and VI-12, VI-21, $ILys^8$ and $IOrn^8$ were about equivalent. For the other three pairs, the analogs with $ILys^8$ were more active, but the differences were not large. The largest difference was for the pair with Val^7, where the $ILys^8$-analog VI-14 showed 90% AOA at 0.5ug vs. 57% for the $IOrn^8$-analog VI-20.

Analog VI-19 was tested, *in vitro*, for histamine release. The ED_{50} value is 42±3.1; i.e., the histamine release is 2-fold that of the analog with one more CH_2 unit. The wheal response did not change conspicuously except for the $Aile^7$ and $IOrn^8$ analog 21 which had the low value of 78.6±4.5 compared to the ILys analog 12 which had 97.9±2.9.

Table VII shows the duration of action of Antide and two analogs. When Antide was injected 44 hours before 50 ng of [D-Qal^6]-LHRH (Qal represents 3(3-quinolyl)alanine), a superagonist, at doses of 3, 10, and 30ug, significant reductions in serum LH were observed at the two higher doses. The LH decreased from 113±11 to 46±12 and 5±0.7 ng/ul. Serum FSH was also decreased, most significantly from about 300 to about 300 ng/ul at 30ug.

Analog VII-24, [Tyr^5]-Antide, and analog IV-6 were similarly injected 24 hours before the agonist. Analog VII-24 showed high activity, reducing the LH level to 19±4, 3±0.4 and 0.3±0.03 ng/ul at doses of 3, 10, and 30ug, respectively. The corresponding figures for analog IV-6 are 42±7, 15±3, and 3.4±2 ng/ul. This is interesting since in the antiovulatory assay analog IV-6 is considerably more potent, 73% at 0.25 ug vs. 45% at 0.5 ug. Perhaps, analog IV-6 is enzymatically degraded faster than analog VII-24. The long duration of action of these analogs s.c. may also be due to "depot" effects at the site of injection.

Table VIII shows the duration of action of Antide after oral administration. Forty-eight hours after administration of 100 or 300ug dose levels of Antide, there were significantly reduced levels of LH which had been released by 5 ng of [D-Qal^6]-LHRH s.c. Reductions from 21±3 to 4±0.8 and 8±2 ng/ul, respectively, were

observed. The results are about the same in the -24 hour experiment (9±2 and 6±0.3 ng/ul). Antide appears to possess considerable resistance towards degrading enzymes. When Antide was given 2 hours before the agonist, a strong decrease in LH levels was observed. At a dose of 30ug, a significant lowering of the LH level to 6±1 ng/ul was seen. At 100 and 300ug, the levels were 1±0.3 and 0.4±.4 ng/ul, i.e., very low levels. When 10 ng of agonist was used, the results are qualitatively very similar.

For comparison, the last three entries in Table VIII are from experiments with [N-Ac-D-pClPhe^{1,2},D-Trp^{3},D-Arg^{6},D-Ala^{10}]-LHRH, VIII-25, an analog that has been reported to have oral activity, (16). These data show that Antide is more active than VIII-25, since a dose of 30ug given 2 hours before the agonist reduced the LH level from 44±4 to 22±4 ng/ul ($p<0.01$). The value for analog VIII-25 is 39±6 (NS). At 100 ug, the corresponding numbers are 7±3 ($p<0.001$) and 26±7 ($p<0.05$). The FSH levels were, in general, lowered when Antide was administered at -2 hours at 100 or 300ug dose levels.

The results in Table IX show that there is no significant difference between administration of Antide in water or in corn oil.

Antide has also been tested orally in the antiovulatory assay (Table X). The AOA values at 300, 600, and 1200ug dose levels are 18, 73, and 100% respectively. Expressed as rats ovulated/total rats, the numbers are 9/11, 3/11, and 0/11. For analog VIII-25, the numbers 9/11, 4/11, and 0/11 have been reported at dose levels of 500, 1000, and 2000ug, respectively, (16). Antide was about twice as active as analog VIII-25.

Table XI shows a comparison of the oral activities of Antide and four analogs. One was as active as Antide, one was considerably less active and two were less active at low doses (30 and 100ug) and about as active at 300ug.

After a 15 ng s.c. dose of [D-Qal^6]-LHRH, the LH level rose to 91 ± 4.6 ng/ul. At oral dose levels of 30, 100, and 300ug of Antide, reduced levels of LH of 75 ± 3, 20 ± 4, and 5 ± 1 ng/ul, respectively, were recorded. Analog 4 with $PicLys^5$, and D-$PACAla^6$ showed no significant reduction of LH at 30 and 100ug levels, but there was a reduction to 51 ± 6 ng/ul at a 300ug dose.

Analog V-12 with $PicLys^5$, D-$PicLys^6$, and $Aile^7$ and analog IV-6 with $PicLys^5$, *cis*-D-$PzACAla^6$ are less active than Antide at 30 and 100ug, but were equally active at 300 ug. Both of these peptides were substantially more active than Antide in the s.c. antiovulatory assay.

Analog 26 was equipotent with Antide. This is not suprising since the only structural difference between these analogs is a pyrazine instead of a pyridine moiety in the N^E-acyl group of the D-Lys^6 residue.

Table XI and XII also shows results with Antide, for example, when 50 ng of the agonist was used. Comparison of these results with the data from the experiments using 15 ng of agonist shows a dose-response relationship which is expected from competitive antagonism. Using 15 ng of agonist, 100 and 300ug of Antide reduced the LH level from 115 ± 15 ng/ul to 20 ± 4 and 5 ± 1 ng/ul respectively, while in the experiments using 50 ng of agonist, 300 and 900ug of Antide reduced the LH to the same level (19 ± 3 and 5.3 ± 1.2 ng/ul).

Table XIII shows the biological effects of Antide in a dispersed pituitary cell culture system.

The structures and biological activites of certain preferred LHRH analogs inhibiting more than 50% of ovulatory activity at a dose of 0.25 ug are shown in Table XIV.

It is proposed that Antide and other antagonists of the present invention may be utilized to induce a state of reversible medical castration that will be of value in the treatment of a rather large number of diseased states such as endometriosis, uterine fibroids and hormonal dependent cancers (prostate, breast). In some patients temporary inhibition of the function of the gonads with Antide, for example, while the patient is receiving chemotherapeutic agents and/or irradiation may prevent or minimize adverse effects of these agents on the gonads and thus help to preserve future fertility. Therapeutic examples would be irradiation during bone marrow transplantation, cervical carcinoma, metastatic thyroid and uterine carcinoma, possibly thyrotoxicosis, etc. during chemotherapy for disseminated lupus erythematosus and certain stages of organ transplantation. More physiological usages of the antagonists of the present invention such as Antide would be to inhibit fertility in both females and males.

More unique possible usages of Antide or other decapeptides of the present invention would be to modify sexual behavior during select disease states. Such disease states could involve patients with AIDS, the aggressive behavior of sex offenders in prisons or aggressive adolescents confined to corrective institutions. It is also possible is that high serum gonadotrophin levels of post-menopausal women may induce functional abnormalities in fat cells that cause weight

gain or in bone cells that play a role in accelerated osteoporosis. These functional abnormalities could potentially be reduced with administration of Antide by inhibiting the high LH and/or FSH level in serum of post menopausal women.

Selective LH-RH antagonists mainly with charged amino acid substitutions in position 6 and/or 8 of the decapeptides probably stimulate histamine release by a direct effect on mast cells to release histamine while other LH-RH antagonists like Antide do not. It is thus proposed that the mast cell-stimulating antagonists applied locally to wounds of the skin may accelerate healing while non-histamine stimulating antagonists may prevent some of the allergic reactions which occur in humans.

To delay the onset of puberty in short stature children by administration of Antide with and without concommitant administration of GH or GH-releasing peptides is proposed as a unique method to enhance body height. The presence of gonadal hormones fuse the epiphysis of long bone and prevent their further elongation. This approach should extend and augment the use and effectiveness of GH and GH-releasing peptides.

The administration of LH-RH antagonists of the present invention acutely inhibits the function of the gonads within 24 hours. Continuous administration of LH-RH superagonists also inhibits the function of the gonads but this is only after several days of stimulating the gonads to hyperfunction. Such superagonist administration introduces a number of potential undesirable clinical problems in patients with prostate cancer, endometriosis, uterine fibroids as well as with sex offenders and those subjected to a temporary induction of medical castration.

For these reasons it is proposed that LH-RH antagonists will be more desirable agents than LH-RH agonists for introducing a reversible state of medical castration. At the diagnostic level, such as differentiating the anatomic source of steroid secretion from the adrenal versus the ovary or to reveal the degree of calcium excretion dependency on gonadal steroid hormones, the rapid onset of inhibiting gonadal function with LH-RH antagonists makes them an unequivocally superior agent over LH-RH agonists. It is proposed that, in every clinical situation where LH-RH superagonists have been utilized to inhibit gonadal function, the LH-RH antagonists will be the agents of choice.

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Changes may be made in the particular amino acid or derivatives and their assembly described herein or in the steps or the sequence of steps of the method described herein without departing from the concept and scope of the invention as defined in the following claims.

CLAIMS:

1. A decapeptide having antiovulatory activity comprising Ser^4, $PicLys^5$ and $D\text{-}PicLys^6$.

2. A decapeptide having antiovulatory activity comprising $N\text{-}Ac\text{-}D\text{-}2\text{-}Nal^1$, $D\text{-}pClPhe^2$, Ser^4, $D\text{-}PicLys^5$ and Pro^9.

3. A decapeptide having antiovulatory activity comprising $N\text{-}Ac\text{-}D\text{-}2\text{-}Nal^1$, $D\text{-}pClPhe^2$, $D\text{-}3\text{-}Pal^3$, Ser^4, $D\text{-}PicLys^6$, Pro^9 and $D\text{-}Ala^{10}$.

4. A decapeptide having antiovulatory activity comprising $N\text{-}Ac\text{-}D\text{-}2\text{-}Nal^1$, $D\text{-}pClPhe^2$, $D\text{-}3\text{-}Pal^3$, Ser^4, $NicLys^5$, Pro^9 and $D\text{-}Ala^{10}$.

5. A decapeptide having antiovulatory activity comprising $N\text{-}Ac\text{-}D\text{-}2\text{-}Nal^1$, $D\text{-}pClPhe^2$, $D\text{-}3\text{-}Pal^3$, Ser^4, Leu^7, Pro^9 and $D\text{-}Ala^{10}$.

6. A decapeptide having antiovulatory activity comprising $N\text{-}Ac\text{-}D\text{-}2\text{-}Nal^1$, $D\text{-}pClPhe^2$, $D\text{-}3\text{-}Pal^3$, Ser^4, Leu^7, Pro^9 and $D\text{-}Ser^{10}$.

7. A decapeptide having antiovulatory activity comprising $D\text{-}pClPhe^2$, Pro^9 and $D\text{-}Ala^{10}$.

8. A decapeptide having antiovulatory activity comprising D-pClPhe^2, Pro^9 and Ser^{10}.

9. A decapeptide having antiovulatory activity comprising N-Ac-D-2-Nal^1, D-pClPhe^2, D-3-Pal^3, NicLys^5, D-NicLys^6, ILys^8 and D-Ala^{10}.

10. A decapeptide having antiovulatory activity comprising N-Ac-D-2-Nal^1, D-pClPhe^2, D-3-Pal^3, NicLys^5, D-NicLys^6, ILys^8 and D-Ala^{10}.

11. A decapeptide having antiovulatory activity comprising N-Ac-D-2-Nal^1, D-pClPhe^2, D-3-Pal^3, PicLys^5, D-PicLys^6, ILys^8 and D-Ala^{10}.

12. A decapeptide having antiovulatory activity comprising N-Ac-D-2-Nal^1, D-pClPhe^2, D-3-Pal^3, NicLys^5, D-NicLys^6, IOrn^8 and D-Ala^{10}.

13. A decapeptide having antiovulatory activity comprising N-Ac-D-2-Nal^1, D-pClPhe^2, D-3-Pal^3, PicLys^5, D-PicLys^6, IOrn^8 and D-Ala^{10}.

14. A decapeptide having antiovulatory activity comprising N-Ac-D-2-Nal^1, D-pClPhe^2, D-3-Pal^3, MNicLys^5, D-MNicLys^6, IOrn^8 and D-Ala^{10}.

15. A decapeptide having antiovulatory activity comprising N-Ac-D-2-Nal^{1}, D-pClPhe^{2}, D-3-Pal^{3}, PzcLys^{5}, D-PzcLys^{6}, IOrn^{8} and D-Ala^{10}.

16. A decapeptide having antiovulatory activity comprising N-Ac-D-pClPhe^{1}, D-3-Pal^{3}, Tyr^{5}, D-NicLys^{6} and ILys^{8}.

17. A decapeptide having antiovulatory activity comprising N-Ac-D-Cl_2Phe^{1}, D-3-Pal^{3}, Tyr^{5}, D-NicLys^{6} and ILys^{8}.

18. A decapeptide having antiovulatory activity comprising acylated Lys^{5}, D-acylated Lys^{6} and N-alkylated diamino acid^{8}.

19. A decapeptide having antiovulatory activity comprising NicLys^{5}, D-NicLys^{6} and ILys^{8}.

20. A decapeptide having antiovulatory activity comprising PicLys^{5}, D-PicLys^{6} and ILys^{8}.

21. A decapeptide having antiovulatory activity comprising NicLys^{5}, D-NicLys^{6} and IOrn^{8}.

22. A decapeptide having antiovulatory activity comprising PicLys^{5}, D-PicLys^{6} and IOrn^{8}.

23. A decapeptide having antiovulatory activity comprising $MNicLys^{5}$, $D\text{-}MNicLys^{6}$ and $IOrn^{8}$.

24. A decapeptide having antiovulatory activity comprising $PzcLys^{5}$, $D\text{-}PzcLys^{6}$ and $IOrn^{8}$.

25. A decapeptide having antiovulatory activity comprising Tyr^{5}, $D\text{-}NicLys^{6}$ and $ILys^{8}$.

26. A decapeptide having antiovulatory activity comprising Tyr^{5}, $D\text{-}NicLys^{6}$ and $IOrn^{8}$.

27. A decapeptide having antiovulatory activity comprising $N\text{-}Ac\text{-}D\text{-}2\text{-}Nal^{1}$, $D\text{-}pClPhe^{2}$, $D\text{-}3\text{-}Pal^{3}$, Ser^{4}, $NicLys^{5}$, $D\text{-}NicLys^{6}$, Leu^{7}, $ILys^{8}$, Pro^{9} and $D\text{-}Ala^{10}NH_{2}$.

28. A decapeptide having antiovulatory activity comprising $N\text{-}Ac\text{-}D\text{-}2\text{-}Nal^{1}$, $D\text{-}pClPhe^{2}$, $D\text{-}3\text{-}Pal^{3}$, Ser^{4}, $PicLys^{5}$, cis $D\text{-}PzACAla^{6}$, Leu^{7}, $ILys^{8}$, Pro^{9} and $D\text{-}Ala^{10}NH_{2}$.

29. A process for inhibiting ovulation in an animal comprising administering to said animal a decapeptide having the structure: $N\text{-}Ac\text{-}D\text{-}2\text{-}Nal^{1}$, $D\text{-}pClPhe^{2}$, $D\text{-}3\text{-}Pal^{3}$, Ser^{4}, $NicLys^{5}$, $D\text{-}NicLys^{6}$, Leu^{7}, $ILys^{8}$, Pro^{9} and $D\text{-}Ala^{10}NH_{2}$.

30. A process for inhibiting ovulation in an animal comprising administering to said animal a decapeptide having the structure: $N\text{-}Ac\text{-}D\text{-}2\text{-}Nal^{1}$, $D\text{-}pClPhe^{2}$, $D\text{-}3\text{-}Pal^{3}$,

Ser^4, $PicLys^5$, cis $D\text{-}PzACAla^6$, Leu^7, $ILys^8$, Pro^9 and $D\text{-}Ala^{10}NH_2$.

31. A process for inhibiting the onset of puberty in an animal comprising administering to said animal a decapeptide having the structure: $N\text{-}Ac\text{-}D\text{-}2\text{-}Nal^1$, $D\text{-}pClPhe^2$, $D\text{-}3\text{-}Pal^3$, Ser^4, $NicLys^5$, $D\text{-}NicLys^6$, Leu^7, $ILys^8$, Pro^9 and $D\text{-}Ala^{10}NH_2$.

32. A process for inhibiting the sexual impetus of an animal comprising administering to said animal a decapeptide having the structure: $N\text{-}Ac\text{-}D\text{-}2\text{-}Nal^1$, $D\text{-}pClPhe^2$, $D\text{-}3\text{-}Pal^3$, Ser^4, $NicLys^5$, $D\text{-}NicLys^6$, Leu^7, $ILys^8$, Pro^9 and $D\text{-}Ala^{10}NH_2$.

33. A process for altering the gonadal function of an animal comprising administering to said animal a decapeptide having the structure: $N\text{-}Ac\text{-}D\text{-}2\text{-}Nal^1$, $D\text{-}pClPhe^2$, $D\text{-}3\text{-}Pal^3$, Ser^4, $NicLys^5$, $D\text{-}NicLys^6$, Leu^7, $ILys^8$, Pro^9 and $D\text{-}Ala^{10}NH_2$.

34. A process for inhibiting the growth of hormone-dependent tumors in an animal comprising administering to said animal a decapeptide having the structure: $N\text{-}Ac\text{-}D\text{-}2\text{-}Nal^1$, $D\text{-}pClPhe^2$, $D\text{-}3\text{-}Pal^3$, Ser^4, $NicLys^5$, $D\text{-}NicLys^6$, Leu^7, $ILys^8$, Pro^9 and $D\text{-}Ala^{10}NH_2$.

35. A process for lowering LH and FSH levels in serum of post-menopausal woman comprising administering to said woman a decapeptide having the structure: $N\text{-}Ac\text{-}D\text{-}2\text{-}Nal^1$,

D-pClPhe^{2}, D-3-Pal^{3}, Ser^{4}, NicLys^{5}, D-NicLys^{6}, Leu^{7}, ILys^{8}, Pro^{9} and D-Ala^{10}NH$_{2}$.

TABLE I. ANTAGONISTS OF LHRH BASED UPON

$[(\ )^1, D\text{-}pClPhe^2, (\ )^3, Ser^4, (\ )^5, (\ )^6, Leu^7, (\ )^8, Pro^9, D\text{-}Ala^{10}]\text{-}NH_2$

NO.	IBR #	Compound ( )^1	( )^3	( )^5	( )^6	( )^8	AOA %/µg 0.5	1.0	2.0	Wheal Area mm^2/10µg	E_D50 µg/ml
		ANALOGS WITH D-NICLYS IN POSITION 6									
1.	22396	N-Ac-D-2-Nal	D-3-Pal	Tyr	D-NicLys	Arg	60	100	--	85	39.2±7
2.	24753	"	"	"	"	Me_3Arg	--	--	--		39.9±7
3.	24825	"	"	"	"	Lys	--	27	--	119.5±3.2	
4.	24315	"	"	"	"	ILys	45	89	100	79.0±9.2	133±22
5.	24443	"	"	"	"	Me_2Lys	--	90	100	122.7	18.4
6.	24748	"	"	"	"	Orn	--	--	67	129.4±3.3	19.3
7.	24756	"	"	"	"	IOrn	22	71	--	92.2±2.9	>300
8.	24199	"	"	Arg	"	Arg	0	42	--	146.8	
9.	24446	"	D-Tyr	"	"	"	33	--	--	113.2±5.6	1.73
10.	25335	"	D-3-Pal	"	"	ILys	43	17	--	196.9±4.2	4.3±0.52
11.	24931	"	"	Me_3Arg	"	"	--	--	44	140±7.0	
12.	25506	"	"	Dpo	"	"	56	--	--	110±3	
13.	24543	"	"	ILys	"	"	--	89	--	132.7±0	20.3
14.	24545	"	"	His	"	Arg	--	89	--	139.7±0	
15.	24593	"	"	3-Pal	"	"	--	100	--	146.4±3.6	
16.	25383	"	"	"	"	ILys	--	75	--	132.8±6.0	86±28*
17.	25384	"	"	"	"	IOrn	--	100	--	139.9±7.2	55±13*
18.	25144	"	"	Ile	"	ILys	--	82	--	147.7±7.1	324±20
19.	25145	"	"	"	"	IOrn	--	55	--	116.5±8.7	151±75
20.	25333	"	"	NicOrn	"	"	--	73	--	113.6±10.9	57±13
21.	25509	"	"	DMGLys	"	ILys	20	--	--	110±3	34±1.1
22.	25510	"	"	PicLys	"	"	64	100	--	116±3.3	39±1.0
23.	25337	N-Ac-D-pClPhe	"	Tyr	"	"	--	100	--	139.9±7.2	198±33*
24.	25338	N-Ac-D-Cl_2Phe	"	"	"	"	0	89	--	103.9±5.3	311±65*

ANALOGS WITH NICLYS IN POSITION 5

25.	22495	N-Ac-D-2-Nal	D-3-Pal	NicLys	D-3-Pal	Arg	0	--	--	112	
26.	24544	"	"	"	D-His	"	--	100	--	146.7±3.6	
27.	24754	"	"	"	D-ILys	"	--	56	73	196.9±4.1	
28.	25334	"	"	"	D-Dpo	ILys	40	100	--	165.2±6.7	6.7±2.2
29.	25332	"	"	"	D-BzLys	"	--	50	--	119.6±6.7	>300
30.	25507	"	"	"	D-Et$_2$hArg	"	--	67	--	123±5.8	
31.	25589	"	"	"	D-PicLys	"	36	--	--	120±7	60±1.4
32.	25588	"	"	"	D-AnGlu	"	--	67	--	113±7	>300
33.	25647	"	"	"	trans-D-NACAla	"	--	70	--	119.5±3.2	
34.	25648	"	"	"	cis-D-NACAla	"	--	100	--	113.6±10.9	37±1.1
35.	25591	"	"	"	D-Me$_2$Lys	"	--	82	--	111±2	262±23
36.	25649	"	"	"	D-PzcLys	"	78	92	--	122.2±5.1	

ANALOGS WITH NICLYS IN POSITION 8

37.	24749	N-Ac-D-2-Nal	D-3-Pal	Tyr	D-Arg	NicLys	--	--	88	136.3±6.8	14.2
38.	24771	"	"	Arg	D-3-Pal	"	0	--	--	99.0±10.3	
39.	24824	"	"	Tyr	D-ILys	"	--	--	100	122.8±5.8	

ANALOGS WITH NICLYS AND D-NICLYS
IN POSITIONS 5, 6 OR IN POSITION 8, 6 OR IN POSITONS 3, 6

40.	24594	N-Ac-D-2-Nal	D-3-Pal	NicLys	D-NicLys	Arg	22	100	--	126.2±8.8	
41.	24987	"	"	"	"	Me$_3$Arg	--	--	100	150.9±14.0	
42.	25143	"	"	"	"	Dpo	--	18	--	113.6±11.1	<300
43.	24542	"	"	"	"	ILys	36	100	100	132.7±0	300
44.	24933	"	"	"	"	IOrn	88	100	--	136.0±3.4	206±64
45.	25078	"	"	"	"	CypLys	--	64	--	147.0±7.1	171±49
46.	24540	"	"	Tyr	"	NicLys	--	0	--	82.6±2.8	300
47.	24745	"	"	His	"	"	--	--	18	136.3±6.8	
48.	24746	"	"	ILys	"	"	--	30	--	132.8±5.9	
49.	24597	"	D-NicLys	Tyr	"	Arg	--	89	--	101.0±6.0	

3/24

MISCELLANEOUS ANALOGS

NO.	IBR #	Compounds ( )[1]	( )[3]	( )[5]	( )[6]	( )[8]	AOA %/µg 0.25	0.5	1.0	2.0	10.0	Wheal Area $mm^2/10\mu g$	E_D50 µg/ml
50.	24596	N-Ac-D-2-Nal	D-3-Pal,	NicLys,	D-NicLys,	NicLys	--	--	0	--	--	122.8±5.7	
51.	24934	"	"	"	NicLys	Ilys	--	--	--	--	8	123±5.9	>300
52.	25146	"	"	INicLys	D-INicLys	"	--	63	91	--	--	140.3±13.9	15±8.2
53.	25147	"	"	PicLys	D-PicLys	"	40	100	90	--	--	123.0±0	93±28
54.	25385	"	"	Arg	D-BzLys	"	--	--	63	--	--	169.0±7.7	8.7±3*
55.	25386	"	"	MNicLys	D-MNicLys	"	--	56	100	--	--	126.1±6.7	>300*
56.	25508	"	"	DMGLys	D-BzLys	"	--	--	100	--	--	136.6.7	24±0.3
57.	25650	"	"	PzcLys	D-PzcLys	"	--	17	--	--	--	110.2±8.1	288±30

*In this test series, the standard compound had an E_D50 value of 0.46 instead of the usual 0.1 -0.2.

TABLE II

ANALOGS WITH $PicLys^5$, $D\text{-}PicLys^6$

	IBR #	Sequence									
58.	26100	N-Ac-D-Cl_2Phe,	D-pClPhe,	D-3-Pal,	Ser,	PicLys,	D-PicLys,	Leu,	ILys,	Pro,	D-Ala-NH_2
59.	25807	N-Ac-D-2-Nal	D-Cl_2Phe	"	"	"	"	"	"	"	"
60.	26364	"	D-3-Pal	D-pClPhe	"	"	"	"	IOrn	"	"
61.	26119	"	D-pClPhe	D-3-PzAla	"	"	"	"	"	"	"
62.	26177	"	"	D-Trp	"	"	"	"	ILys	"	"
63.	25934	"	"	D-3-Pal	"	"	"	Val	"	"	"
64.	26118	"	"	"	"	"	"	"	IOrn	"	"
65.	25936	"	"	"	"	"	"	Aile,	ILys	"	"
66.	26178	"	"	"	"	"	"	"	IOrn	"	"
67.	25990	"	"	"	"	"	"	Abu	ILys	"	"
68.	26179	"	"	"	"	"	"	"	IOrn	"	"
69.	25935	"	"	"	"	"	"	Trp	ILys	"	"
70.	25988	"	"	"	"	"	"	Nle	"	"	"
71.	25989	"	"	"	"	"	"	Nval	"	"	"
72.	26020	"	"	"	"	"	"	Ile	"	"	"
73.	26099	"	"	"	"	"	"	Ala	"	"	"
74.	26346	"	"	"	"	"	"	Abu	Arg	"	"
75.	25937	"	"	"	"	"	"	Leu	ILys	Pip	"
76.	26019	"	"	"	"	"	"	"	"	Pro,D-Abu-NH_2	
77.	25933	"	"	"	"	"	"	"	Iorn	"	D-Ala-NH_2

Analogs with PicLys^5.

78.	26349	N-Ac-D-2-Nal	D-pClPhe	D-TinGly	Ser	PicLys	c-D-PzACAla	Leu	ILys	Pro	D-Ala-NH_2
79.	26324	"	"	D-3-PxAla	"	"	"	"	"	"	"
80.	25897	"	"	D-3-Pal	"	"	"	"	"	"	"
81.	26181	"	"	"	"	"	"	"	IOrn	"	"
82.	26325	"	"	"	"	"	"	Val	ILys	"	"
83.	26366	"	"	"	"	"	"	Phe	"	"	"
84.	26347	"	"	"	"	"	"	Leu	Arg	"	"
85.	26348	"	"	"	"	"	"	"	ILys	"	D-Ser-NH_2
86.	26383	"	"	"	"	"	"	"	"	"	NHEt
87.	26323	"	"	"	"	"	c-D-PmACAla	"	"	"	D-Ala-NH_2

Analogs with D-PicLys^6

88.	26180	N-Ac-D-2-Nal	D-pClPhe	D-3-Pal	Ser	c-PzACAla	D-PicLys	Leu	ILys	Pro	D-Ala-NH_2
89.	26381	"	"	"	"	HOBLys	"	Abu	"	"	"
90.	26382	"	"	"	"	Cit	"	"	"	"	"
91.	26363	"	"	"	"	Tyr	"	Leu	IOrn	"	"

Analogs with NicLys^5.

92.	25805	N-Ac-D-2-Nal	D-pClPhe	D-3-Pal	Ser	NicLys	t-D-PzACAla	Leu	ILys	Pro	D-Ala-NH_2
93.	25806	"	"	"	"	"	c-D-PzACAla	"	"	"	"
94.	26345	"	"	"	"	"	D-NicLys	NMeLeu	"	"	"
95.	25991	"	"	"	"	"	D-PzcLys	Leu	IOrn	"	"

Miscellaneous Substitutions in Positions 5 and 6.

96.	25808	N-Ac-D-2-Nal	D-pClPhe	D-3-Pal	Ser	MPicLys	D-M-PicLys	Leu	ILys	Pro	D-Ala-NH_2
97.	26322	"	"	"	"	PmcLys	D-PmcLys	"	"	"	"
98.	26326	"	"	"	"	c-PzACAla	c-D-PzACAla	"	"	"	"
99.	26417	"	"	"	"	"	D-3-PzAla	"	Arg	"	"
100.	26418	"	"	"	"	Tyr	c-D-PzACAla	"	ILys	"	"
101.	26365	"	"	"	"	"	"	"	"	"	D-Ser-NH_2

Analogs Being Synthesized at This Time.

102.	N-Ac-D-2-Nal	D-pClPhe	D-3-Pal	Ser	Arg	D-3-Pal	Leu	Arg	Pro	Sar-NH_2
103.	"	"	"	"	PicLys	c-D-PzACAla	"	ILys	"	"
104.	D-pGlu	"	D-Phe	"	Arg	D-3-Pal	"	Arg	"	D-Ala-NH_2
105.	N-Ac-D-2-Nal	"	D-3-Pal	"	c-PzACAla	D-PicLys	Val	ILys	"	

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TABLE III Biological Data.

Analogs with $PicLys^5$,$D\text{-}PicLys^6$

NO.	IBR #	AOA/µg 0.25	AOA/µg 0.5	AOA/µg 1.0	Wheal Area $mm^2/10\mu g$	In Vitro Histamine Release ED_{50} µg/ml ± SEM
58.	26100	-	38	-	116.2±3.7	
59.	25807	-	64	90	139.8±7.1	
60.	26364	12	-	-	116.2±5.5	
61.	26119	-	75	-	103.9±3.4	
62.	26177	-	20	-	71.0±4.3	
63.	25934	43	90	100	97.9±2.9	213 ± 30
64.	26118	-	57	-	119.6±6.6	
65.	25936	43	89	-	97.9±2.9	> 300
66.	26178	-	82	-	78.6±4.5	
67.	25990	36	100	-	91.0±5.4	273 ± 27
68.	26179	-	80	-	101.5±9.3	
69.	25935	-	10	-	78.5±0	
70.	25988	20	77	-	107.0±6.0	
71.	25989	10	100	-	95.3±6.0	
72.	26020	0	-	-	110.7±2.3	
73.	26099	-	60	-	103.9±3.7	
74.	26346	50	88	-	113.2±5.4	
75.	25937	-	0	100	95.0±0	
76.	26019	-	78	-	109.9±3.0	
77.	25933	50	90	100	113.0±0	

					Analogs With PicLys[5]	
78.	26349	0	-	-	84.6±3.9	
79.	26324	22	100	-	127.8±4.9	
80.	25897	73	100	-	122.8±5.7	28 ± 7.5
81.	26181	50	100	-	101.6±2.2	
82.	26325	73	100	-	127.8±4.9	
83.	26366	0	-	-	116.2±3.2	
84.	26347	14	-	-	119.6±8.5	
85.	26348	22	-	-	122.8±5.7	
86.	26383	25	-	-	119.6±6.6	
87.	26323	-	9	-	120.4±4.7	
					Analogs With D-PicLys[6]	
88.	26180	67	90	-	99.5±4.5	
89.	26381	11	-	-	95.1±5.0	
90.	26382	11	-	-	89.5±5.5	
91.	26363	0	-	-	113.2±5.5	
					Analogs With NicLys[5]	
92.	25805	-	67	88	129.6±8.8	
93.	25806	-	-	25	101.7±5.0	
94.	26345	10	-	-	110.5±11.4	
95.	25991	-	44	-	104.3±10.5	
					Analogs With Miscellaneous Substituents in Positions 5 and 6.	
96.	25808	-	67	91	106.2±4.3	
97.	26322	0	-	-	130.2±2.5	
98.	26326	57	100	-	115.5±2.4	
99.	26417	22	-	-	133.2±11.8	
100.	26418	22	-	-	95.0±0	
101.	26365	0	-	-	129.4±3.3	

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TABLE IV

Biological Data for [N-Ac-D-2-Nal^{1},D-pClPhe^{2},D-3-Pal^{3},X^{5},Y^{6},ILys^{8},D-Ala^{10}]-LHRH Analogs

NO.	X	Y	AOA %/µg 0.125	0.25	0.5	1.0	In Vitro Histamine Release ED_{50} µg/ml±SEM	Wheal Area mm^2/10µg
IV-1.	NicLys	<u>trans</u>-D-NACAla	–	–	–	70		119.5±3.2
IV-2.	"	<u>cis</u>-D-NACAla	–	–	50	100	37±1.1	101.8±4.3
IV-3.	PicLys	<u>trans</u>-D-PACAla	–	–	50	–	64±5.4	101.0±3.0
IV-4.	"	<u>cis</u>-D-PACAla	–	–	54	–	41±5.4	123.0±5.0
IV-5.	"	<u>trans</u>-D-PzACAla	–	–	44	–	39±4.4	106.3±4.3
IV-6.	"	<u>cis</u>-D-PzACAla	29	73	100	–	28±7.5	122.8±5.7
IV-7.	NicLys	<u>trans</u>-D-PzACAla	–	–	67	88		129.6±8.8
IV-8.	"	<u>cis</u>-D-PzACAla	–	–	–	25		101.7±5.0
IV-9.	<u>cis</u>-PzACAla	D-PicLys	–	67	90	–		99.5±4.5

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TABLE V

Biological Data for [N-Ac-D-2-Nal^{1},D-pClPhe^{2},D-3-Pal^{3},PicLys^{5},D-PicLys^{6},X^{7},ILys^{8},D-Ala^{10}]-LHRH Analogs.

NO.	X	AOA %/µg 0.25	0.5	1.0	In Vitro Histamine Release ED_{50} µg/ml±SEM	Wheal Area mm^2/10µg
V-10.*	Leu	40	100	90	93±11	123±0
V-11.	Ile	0	-	-		110.7±2.3
V-12.	Aile	43	89	-	>300	97.9±2.9
V-13.	Nle	20	77	-		107.0±6.0
V-14.	Val	43	90	100	213±30	97.9±2.9
V-15.	NVal	10	100	-		95.3±6.0
V-16.	Abu	36	100	-	273±27	91.0±5.4
V-17.	Ala	-	60	-		103.9±3.7
V-18.	Trp	-	10	-		78.5±0

* From Reference 1

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TABLE VI

Biological Data for [N-Ac-D-2-Nal^1,D-pClPhe^2,D-3-Pal^3,PicLys^5,X^6,Y^7,Z^8,D-Ala^{10}]-LHRH Analogs

NO.	X	Y	Z	AOA%/μg 0.25	0.5	1.0	In Vitro Histamine Release ED_{50} μg/ml±SEM	Wheal Area mm^2/10μg
VI-10.*	D-PicLys	Leu	ILys	40	100	90	93±11	123±0
VI-19.	"	"	IOrn	50	90	100	42±3.1	113.0±0
VI-14.	"	Val	Ilys	43	90	100	213±30	97.9±2.9
VI-20.	"	"	IOrn	-	57	-		119.6±6.6
VI-12.	"	Aile	ILys	43	89	-	>300	97.9±2.9
VI-21	"	"	IOrn	-	82	-		78.6±4.5
VI-16.	"	Abu	ILys	36	100	-	273±27	91.0±5.4
VI-22.	"	"	IOrn	-	80	-		101.5±9.3
VI-6.	cis-D-PzACAla	Leu	ILys	73	100	-	28±7.5	122.8±5.7
VI-23.	"	"	IOrn	50	100	-		101.6±2.2

* From Reference 1

TABLE VII

Duration of Action of Antide and Two Analogs Subcutaneously* Administered.

Analog	Injection Time	Dose µg	0 Time ng sc [D-3-Qal^6]-LHRH	+2 hrs LH ng/ml ±SEM	p value	FSH ng/ml ± SEM	p value
-	-	-	-	0.4±0.03	<.001	143±10	<.001
-	-	-	50	113±11	-	2899±387	-
Antide	-44hr	3	50	90±5	NS	2497±155	NS
"	"	10	50	46±12	<.001	1413±230	<.01
"	"	30	50	5±0.7	<.001	311±34	<.001
VII-24†	-24hr	3	50	19±4	<.001	719±123	<.001
"	"	10	50	3±0.4	<.001	289±30	<.001
"	"	30	50	0.3±0.03	<.001	147±10	<.001
IV-6(25897)	"	1	50	91±19	NS	2020±295	NS
"	"	3	50	42±7	<.001	1298±275	<.01
"	"	10	50	15±3	<.001	624+84	<.001

				+2 hrs			
Analog	Injection Time	Dose µg	0 Time ng sc [D-3-Qal^6]-LHRH	LH ng/ml ±SEM	p value	FSH ng/ml ± SEM	p value
"	"	30	50	3.4±2	<.001	273±89	<.001

* Mean of 6 ± SEM

† [Tyr^5]-Antide

TABLE VIII

Duration of Action of Orally Administered Antide and Comparison with

[N-Ac-D-pClPhe^{1,2},D-Trp^{3},D-Arg^{6},D-Ala^{10}]-LHRH (25).*

Antagonist	Time of adm.†† hr	Dose µg	0 Time Agonist† Dose (sc) ng	+2 hours Serum LH ng/ml ± SEM	+2 hours p value	+2 hours FSH ng/ml ± SEM	+2 hours p value
	-	-	-	3±1	<.001	298±20	<.001
	-	-	5	21±3	-	796±102	-
Antide	-48	100	5	4±0.8	<.001	481±27	<.02
"	-48	300	5	8+2	<.01	600±72	NS
"	-24	100	5	9±2	<.01	596±50	NS
"	-24	300	5	6±0.3	<.001	462±54	<.02
"	-2	10	5	19±4	NS	588±70	NS
"	-2	30	5	6±1	<.001	573±67	NS
"	-2	100	5	1±0.3	<.001	320±48	<.01
"	-2	300	5	0.4±0.4	<.001	327±63	<.01
-	-	-	-	3±1	<.001	298±20	<.001
-	-	-	10	44±4	-	1488±168	-
Antide	-48	100	10	18±2	<.001	792±110	<.01
"	-48	300	10	25±3	<.01	1021±202	NS
"	-24	100	10	24±6	<.02	1008±285	NS
"	-24	100	10	25±3	<.01	1119±71	NS
"	-2	10	10	51±8	NS	1729±243	NS
"	-2	30	10	22±4	<.01	1051±141	NS
"	-2	100	10	7±3	<.001	548±83	<.001
"	-2	300	10	0.5±.06	<.001	251±24	<.001

Antagonist	Time of adm.†† hr	Dose µg	0 Time Agonist† Dose (sc) ng	+2 hours Serum LH ng/ml ± SEM	p value	FSH ng/ml ± SEM	p value
VIII-25	-2	10	10	59±11	NS	1794±329	NS
"	-2	30	10	39±6	NS	1470±190	NS
"	-2	100	10	26±7	<.05	1161±277	NS

* Kindly provided by Dr. David Coy

† [D-Qal^6]-LHRH

†† Administered in water

TABLE IX

Oral Activity of Antide. Dependence on Vehicle.

Vehicle	-2 hrs Antagonist Dose µg oral	0 Time Agonist Dose ng sc	+2 hrs LH ng/nl ± SEM	p value	FSH ng/ml ± SEM	p value
water	-	-	1.1±0.1	<.001	243±35	<.001
"	-	50	148±9	-	3041±238	-
"	100	50	44±5	<.001	1372±84	<.001
"	300	50	20±4	<.001	936±150	<.001
"	900*	50	6.3±3	<.001	374±80	<.001
corn oil	-	-	0.8±0.6	<.001	138±6	<.001
"	-	50	115±8	-	2935±133	-
"	100	50	72±12	<.01	2148±234	<.02

Vehicle	-2 hrs Antagonist Dose µg oral	0 Time Agonist Dose ng sc	+2 hrs LH ng/nl ± SEM	p value	FSH ng/ml ± SEM	p value
"	300	50	20±4	<.001	792±137	<.001
"	900	50	7±2	<.001	599±59	<.001

Design: -2 hrs - Antagonist

0 time - [D-3-Qal^6]-LHRH

+2 hrs - Sacrifice

26 day old female rates. Mean of 6 ± SEM

* Diluted 1:1 with 10 mM HOAC:Water (slightly cloudy) 0.1 ml orally, other concentration diluted with water

TABLE X

Oral Activity of Antide in the Antiovulatory Assay.*

Oral Dose µg	AOA % Inhibition (# Ovulated / # Rats)
--	0 (6/6)
300	18 (9/11)
600	73 (3/11)
1200	100 (0/11)

* in 10mM acetic acid:water (1:1)

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TABLE XI

Oral Activity of Antide and Some Analogs.

Antagonist	-2 hrs Dose µg oral	0 Time Agonist Dose ng sc	+2 hrs LH ng/ml ± SEM	p value	FSH ng/ml ± SEM	p value
	-	-	3.4±2.2	<.001	271±56	<.001
	-	15	91±4.6	-	2491±146	-
Antide	30	15	75±3	<.02	1718±223	<.02
"	100	15	20±4	<.001	738±89	<.001
"	300	15	5±1	<.001	472±26	<.001
4	30	15	79±9	NS	1831±249	<.05
"	100	15	76±6	NS	2175±211	NS
"	300	15	51±6	<.001	1404±117	<.001
12	30	15	71±9	NS	1965±256	NS
"	100	15	54±10	<.01	1031±195	<.001
"	300	15	6±1.1	<.001	514±54	<.001
26*	30	15	75±9	NS	2438±207	NS
"	100	15	19±3	<.001	845±149	<.001
"	300	15	6±1.4	<.001	431±22	<.001
6	30	15	77±12	NS	1761±191	<.01
"	100	15	59±12	<.05	1782±388	NS
"	300	15	6.3±1.4	<.001	467±43	<.001
	-	50	115±15	-	2372±126	-
Antide	30	50	93±7	NS	2262±55	NS
"	100	50	49±7	<.001	1345±199	<.001

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Antagonist	-2 hrs Dose µg oral	0 Time Agonist Dose ng sc	+2 hrs LH ng/ml ± SEM	p value	FSH ng/ml ± SEM	p value
"	300	50	19±3	<.001	630±40	<.001
"	900	50	5.3±1.2	<.001	450±48	<.001

Design: -2 hrs - Antagonist
0 Time - [D-3-Qal^6]-LHRH
+2 hrs - Sacrifice

26 day old female rats. Mean of 6 ± SEM
Vehicle - 10 mM HOAC:Water (1:1) 0.1 ml

* [D-N$^\varepsilon$-pyrazinylcarbonyllysyl^6]-Antide.

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TABLE XII

ORAL ACTIVITY OF ANTIDE
At Various Time Schedules and Doses of a LH-RH Superagonist

[NAcD2Nal^{1},DpClPhe^{2},D3Pal^{3},NicLys^{5},DNicLys^{6},ILys^{8},DAla^{10}]LHRH

Antagonist Time adm. (oral) hr	Antagonist Dosage µg	Agonist* Dose (sc) 0 TIME	LH ng/ml ± SEM	p value +2 HOURS	FSH ng/ml ± SEM	p value
--	--	--	3 ± 1	<.001	298 ± 20	<.001
--	--	5 ng	21 ± 3	--	796 ± 120	--
-48	100	5 ng	4 ± 0.8	<.001	481 ± 27	<.02
-48	300	5 ng	8 ± 2	<.01	600 ± 72	NS
-24	100	5 ng	9 ± 2	<.01	596 ± 50	NS
-24	300	5 ng	6 ± 0.3	<.001	462 ± 54	<.02
-2	10	5 ng	19 ± 4	NS	588 ± 70	NS
-2	30	5 ng	6 ± 1	<.001	573 ± 67	NS
-2	100	5 ng	1 ± 0.3	<.001	320 ± 48	<.01
-2	300	5 ng	0.4 ± 0.4	<.001	327 ± 63	<.01
--	--	--	3 ± 1	<.001	298 ± 20	<.001
--	--	10 ng	44 ± 4	--	1488 ± 168	--
-48	100	10 ng	18 ± 2	<.001	792 ± 110	<.01
-48	300	10 ng	25 ± 3	<.01	1021 ± 202	NS

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Antagonist Time adm. (oral) hr	Antagonist Dosage µg	Agonist* Dose (sc) 0 TIME	LH ng/ml ± SEM	p value +2 HOURS	FSH ng/ml ± SEM	p value
-24	100	10 ng	24 ± 6	<.02	1008 ± 285	NS
-24	300	10 ng	25 ± 3	<.01	1119 ± 71	NS
-2	10	10 ng	51 ± 8	NS	1729 ± 243	NS
-2	30	10 ng	22 ± 4	<.01	1051 ± 141	NS
-2	100	10 ng	7 ± 3	<.001	548 ± 83	<.001
-2	300	10 ng	0.5 ± .06	<.001	251 ± 24	<.001
-2	10**	10 ng	59 ± 11	NS	1794 ± 329	NS
-2	30	10 ng	39 ± 6	NS	1470 ± 190	NS
-2	100	10 ng	26 ± 7	<.05	1161 ± 277	NS

* 24270 [D3Qal^{6}]-LHRH

** AH-195-3 NAcDpClPhe^{1,2},DTrp^{3},DAla^{10}-LHRH (Dr. David Coy)

mean of 6 ± SEM

TABLE XIII

Effect of Antide in the
Dispersed Pituitary Cell Culture Assay

Peptide	Dose nM	LHRH nM	RLH ng/ml ±SEM	p value	IDR_{50}	FSH ng/ml ±SEM	p value	IDR_{50}
Control	--	--	10±0.4	--		196±23	--	
LHRH	--	0.1	40±7	<.05		221±18	NS	
	--	0.3	80±1	<.001		562±48	≈.02	
	--	1.0	118±	NA		802±	NA	
	--	3.0	150±1	<.001		646±123	NS	
	--	10.0	141±4	<.001	--	602±26	<.01	--
	--	30.0	152±7	<.01		557±15	<.01	
139-95-20	0.01	3.0	118±11	NS*		546±93	NS*	
	0.03	3.0	117±10	NS	0.26:1	499±26	NS	0.52:1
	0.1	3.0	116±7	<.05		472±59	NS	
	0.3	3.0	107±11	NS		617±73	NS	
	1.0	3.0	80±2	<.001		481±17	NS	
	3.0	3.0	34±2	<.001		233±38	NS	
	10.0	3.0	11±1	<.001		165±21	NS	

* p values vs 3 nM of LHRH

139-95-20 [$NAcD2Nal^{1}$,$DpClPhe^{2}$,$D3Pal^{3}$,$NicLys^{5}$,$DNicLys^{6}$,$ILys^{8}$,$DAla^{10}$]LHRH

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TABLE XIV

LHRH analogs with 50% or more AOA at 0.25 ug

IBR#	Sequence										AOA/0.25	Wheal area	ED
25897	N-Ac-D-2-Nal,	DpClPhe,	D-3-Pal,	Ser,	PicLys,	c-D-PzACAla,	Leu,	ILys,	Pro,	D-Ala-NH_2	73	122.8 ± 5.7	28^{50} ± 7.
26325	"	"	"	"	"	"	Val	"	"	"	73	127.8 ± 4.9	
26180	"	"	"	"	c-PzACAla,	D-PicLys	Leu	"	"	"	67	99.5 ± 4.5	
26326	"	"	"	"	"	c-D-PzACAla	"	"	"	"	57	115.5 ± 2.4	
26181	"	"	"	"	PicLys	"	"	IOrn	"	"	50	101.6 ± 2.2	
*25933	"	"	"	"	"	D-PicLys	"	"	"	"	50	113.0 ± 0	
26346	"	"	"	"	"	"	Abu	Arg	"	"	50	113.2 ± 5.4	

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*Claimed in original

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/02922

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC
IPC4: C 07 K 7/20, A 61 K 37/38,/43

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC4	A 61 K, C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	EP, A1, 81877 (COY, DAVID HOWARD) 22 June 1983, the examples --	7
X	EP, A2, 97031 (SYNTEX) 28 December 1983, see page 15 - page 16 --	5,7
X	EP, A1, 0143573 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 5 June 1985, see page 9 --	7
X	EP, A2, 0162575 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 27 November 1985, see page 23 --	5,7

.../...

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
29th December 1988	27 JAN 1989
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	EP, A2, 0175506 (THE SALKINSTITUTE FOR BIOLOGICAL STUDIES) 26 March 1986, see page 15 --	7
X	EP, A2, 0197798 (ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND) 15 October 1986, see page 5 --	7
X	EP, A2, 0199302 (SYNTEX (U.S.A.) INC.) 29 October 1986, --	5,7
X	EP, A2, 0225746 (THE ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND) 16 June 1987, see page 7 --	7
P,X	EP, A2, 0277829 (SYNTEX (U.S.A.) INC.) 10 August 1988, see page 7 - page 9 --	5,7
X	US, A, 4431635 (DAVID H. COY ET AL) 14 February 1984, EXAMPLES 16,19 --	7
X	US, A, 4444759 (RIVIER ET AL) 24 April 1984, the claims --	7
X	US, A, 4504414 (FOLKERS ET AL) 12 March 1985, table 1 --	5,7
X	US, A, 4647653 (DAVID H. COY) 3 March 1987, --	7
X	J. Med. Chem., Vol. 29, 1986 Jean E. Rivier et al: "New Effective Gonadotropin Releasing Hormone Antagonists with Minimal Potency for Histamine Release in Vitro ", pages 1846-51 see the whole document --	7
X	Endocrine Reviews, Vol. 7, No. 1, 1986 (USA) Marvin J. Karten and Jean E. Rivier: "Gonadotropin-Releasing Hormone Analog Design. Structure- Function Studies Toward the Development of Agonists and Antagonists:Rationale and Perspective ", pages 44-66, pages 54-57; page 60 --	7

.../...

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
P,X	Biochemical and biophysical research communications, Vol. 148, No. 2, 1987 Anders Ljungqvist et al: "Design, synthesis and bioassays of antagonists of LHRH which have antiovulatory activity and release negligible histamine ", pages 849-56 see the whole document --	1-5,7,9-12,16-21, 25-27
P,X	Proc.Natl.Sci., Vol. 85, 1988 (USA) S. Bajusz et al: "Highly potent antagonists of luteinizing hormone- releasing hormone free of edematogenic effects ", pages 1637-41 see the whole document --------	7

International Application No. PCT/US88/02922

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [x] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [x] Claim numbers 29-35 because they relate to subject matter not required to be searched by this Authority, namely:

   Method for treatment of the human or animal body by therapy. Rule 39(iv).

2. [ ] Claim numbers ...., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers........, because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/US 88/02922

SA 24550

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The members are as contained in the European Patent Office EDP file on 02/11/88
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		AU-D- 91025/82	16/06/83
EP-A2- 0097031	28/12/83	AU-D- 15674/83	15/12/83
		JP-A- 59062556	10/04/84
		AU-A- 569036	21/01/88
		AU-D- 79418/87	21/01/88
		US-A- 4481190	06/11/84
		US-A- 4581169	08/04/86
		US-A- 4698442	06/10/87
		US-A- 4667014	19/05/87
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		US-A- 4317815	02/03/82
		EP-A-B- 0041286	09/12/81
		JP-A- 57014568	25/01/82
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		AT-E- 8988	15/09/84
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 4504414	12/03/85	None	
US-A- 4647653	03/03/87	JP 61210098	18/09/86

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82